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RELATIONSHIP OF SUGAR MAPLE DECLINE AND CORRESPONDING CHEMICAL CHANGES IN XYLEM SAP CARBOHYDRATES MICRONUTRIENTS AND TRACE ELEMENTS

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INTRODUCTION

Irrespective of the aetiology of the maple decline syndrome, loss of vigor, biomass production and decrease in sap production have been equated with decline. Although these symptoms are physical indications of maple decline, the biochemical responses may well occur long before their manifestation. Various cell types of the conducting xylem of sugar maple trees function in the circulation, storage, metabolism, mobilization and assimilation of organic substances (Kozlowski 1971). Xylem sap also contains appreciable quantities of carbohydrates in the soluble form along with various nutrients (Wilson 1970). These chemicals could, therefore, be studied to see if these are different in healthy and declining trees. We had earlier proposed that at the present stage of our knowledge of sugar maple decline, a useful approach might be to study the chemical changes occurring in sap in trees ranging from healthy to seriously in decline. The long-term objective being that if we could index the chemical change in sap matrix of declining maple trees, relationships could then be correlated with a physical assessment criterion, Decline Index (DI), recently formulated by Ontario Ministry of the Environment (MOE) and is currently being used in many related studies. It might be possible to index these trees chemically much earlier than the onset of the physical decline, thereby facilitating the "early warning" syndrome of maple decline. The focus of this research, therefore lies in chemical analyses of sap matrix. This pioneering approach has not yet been pursued by any other research group involved in sugar maple decline research.

METHODOLOGY

1. EXPERIMENTAL DESIGN

The sampling unit of this study is an individual (healthy/declining) tree of any of the five sites selected in a NE to SW directional gradient of southern Ontario. Ten sugar maple trees, differeing in physical health (as determined by DI) and site characteristics (mainly pedological and stand) are selected each from five sites varying in their overall decline status. These sites (namely, A061, A062, A045, A073 and A020) were mapped out in 1986 as part of the Hardwood Decline Survey conducted by MOE. One of these sites has been demarcated as a "base-line site" (site A020), which will provide a stastically acceptable pattern of variability in levels of sap chemicals. Fifty trees at various stages of decline have been marked for study at this site. This site will further provide data to study the extent of intra-site variability in these trees. All sites selected have been unmanaged for at least past 17 years, contain at least 50% sugar maple trees, and are situated on the Crown Land, basically undisturbed by any man-made disturbances.

Xylem sap at breast heights (1.3 meters) of selected trees have been collected at both early and late sap flow seasons in March and April months of 1988, respectively. Stem tissue will be collected in early Fall along with the soil from the rooting zone of each tree. At the time of stem and soil collection, the decline status will be assessed with the physical parameters set forth by MOE. Trees are selected so that the healthy trees have

DI of <10.00 and declining trees are branded DI of >18-20. The major response variables selected for this study include Total sugars, major carbohydrates like Sucrose, Glucose, Fructose, Galactose, Raffinose, Stachyose, Arabinose and Mannose and major inorganic elements, for example, macronutrients (Na, K, P, Ca, S, and Mg), micronutrients (B, Cu, Fe, Mn, Zn) and other elements (Al, Co, Cd, N, Pb, Si, Cr, As, Se, Sb, V, Mo, Ni, and Ba)

2. SAP COLLECTION

For sap collection, aseptic conditions were used to avoid metal contamination which might interfere with the trace elemental analyses. A custom designed polyethylene plastic suction apparatus was designed for the extraction of xylem sap in maple trees in the field by MOE and used for the extraction of sap samples in these sites. A two inch size of hole was drilled by an automatic drill attached onto a chain saw at the south side of the breast height of the trees. A maximum pressure of 120 psi, applied by hand-held lysimeter pump, was enough to yield sufficient amount of sap (30-100) from each tree in 5-20 minutes. In 1988, a total of 180 sap samples were collected (90 early sap season samples and 90 late season samples from 90 trees). The total volume of collected samples varied between 25-100 mL. Each extracted sap sample was aliquoted and distributed equally in 4 sterile plastic vials (30 mL. capacity). The samples were kept cold in the field and frozen at -15^{0}C as soon as possible. The frozen samples were thawed and diluted with deionized water (10-1000 X) prior to analyses.

3. ANALYTICAL PROCEDURES

A. Analyses of Carbohydrates and their derivatives

Simple sugars and di- and oligo-saccharides are being analysed by High Pressure Liquid Ion Exchange Chromatography (HPLIC), a new technique marketed recently by Dionex Corporation (Sunnyvale, California), as BioLC Series 4000i. This is a new chromatographic analysis method utilizing anion exchange separation of carbohydrates with pulsed amperometric detection. Compared to older chromatographic methods with refractive index and ultraviolet detection, anion exchange with pulsed amperometric detection provides specificity, selectivity, sensitivity and reliability. In our laboratory the chromatograph has been interfaced with a Shimadzu CR5A integrator (Shimadzu Corp., Japan). Ultrapure carbohydrate standards (Pfanstiehl Lab. Inc., Illinois, USA) with a detection limit as low as 30 ppb for monosaccharides and 100 ppb for polysaccharides can be separated as anions with a concentration elution gradient program of sodium hydroxide. Retention times and selectivity of the analytes were controlled by varying eluent strength. The separated carbohydrates were detected electrochemically by oxidation of a gold electrode. This potent method of direct separation of carbohydrates offers a timely option to replace other cumbersome and unreliable analytical methods (e.g. HPLC utilizing RI and low UV-detection and GC after derivitization of samples) of carbohydrate analyses. These latter methods were tried earlier to establish the ideal conditions for sap samples with limited success. On the other hand, we have found the Ion Exchange Chromatography to be sensitive (30-100 ppb), linear (upto 600 ppm concentration), reproducible (peak heights vary less than 1% after multiple injections of single sample) and in control of retention at both isocratic and gradient elutions of a sample.

After standardizing the system for representative sugars, detailed carbohydrate analyses of sap samples are now in progress and some of the results will be presented in the

Conference. Samples are being analysed in a calibration analytical run of an elution gradient method in combination with a post-column addition of the higher molarity of the eluent used for the carbohydrate standards and sap samples. A computer program to calculate the concentrations of known sugars based on the absolute normalization method has been developed and is now being used to analyse the field sap samples. At present we have completed a preliminary chromatographic analyses of six known sugars (Arabinose, galactose, glucose, fructose, xylose and Sucrose) in 100 sap samples. Other samples are currently being analysed. These data indicate differences in the nature and amount of sugars in trees based on their decline characteristics. We will be characterising the observed "unknown" peaks in certain sap samples once the preliminary analyses of all the samples is completed. Later on silyl derivatives of sugars will be used for detection and quantitation of sugar derivatives by Gas Chromatography. Sugars of unknown structures will be analysed by HPLC-Mass Spectroscopy. Additional use of ^{13}C NMR might be required for complex sugars. Separation of minor components for optional study, that is, phenols, peptides, aminonitrogens, proteins, organic acids, amino acids (AA's) etc., will be followed by the amino acid analysis using the AA analyser. An attempt will be made to analyse AA's in the GC with a special N-P detector.

B. Physico-chemical Analyses

Some other physico-chemical properties of carbohydrates e.g. pH measurements, refractive indices and optical rotations, have been utilised for gross estimation of sugar content and its changes in sap samples. Data for all these variables have been collected and are in the process of being analysed. These variables will provide a secondary support for the final carbohydrate determination.

C. Trace Elemental Analyses

Flash freeze-dried sap samples (12-25 microgram/mL) in 6% nitric acid matrix have been analysed for macronutrients (Na, K, P, Ca, S, and Mg), some micronutrients (B, Cu, Fe, Mn, Zn) and other elements e.g. Al, Co, Cd, N, Pb, Si, Cr, As, Se, Sb, V, Mo, Ni, and Ba, by Induced Coupled Argon Plasma Spectroscopy (ICAP). Sodium and Potassium analyses of freeze-dried sap samples were analysed by Atomic absorption Spectroscopy. A preliminary analysis of the 24 elements shows that there is a trend of increasing amounts of Al, and Mn and decreasing amounts of Ca, Mg, P, and Fe, when sap of healthy trees was compared with that of declining trees. Details of these results will be presented during the meeting.

4. DATA ANALYSIS

At present the analytical data are being input in IBM microcomputer programs (Mainly Reflex, Lotus, Dbase III plus and SAS-PC). Once completed, the stored data will be retrieved for computational purposes. Prior to analyses, data in either %age or ppm units will be tested for normality (by univariate statistics), homoscadaticity (by t-tests) and independence of observations (by scatter plots). Appropriate transformations (e.g. log, square root, logistic transfer etc.) will be applied to data to satisfy these assumptions for detailed data analyses to follow. Differences in tree-health parameters (healthy versus declining) and chemical components will be analysed by either a conventional one-way analysis of variance, in conjunction with t-tests (used as a means separation test), or a student t-test (Snedecor and Cochrane 1982). Correlating response variables will be detected by correlation matrices. If response variables do not correlate, analyses of

variance with individual chemical concentrations will be done, otherwise correlating variables will be grouped to do multivariate analyses of variance (MANOVA) in a factorial design. General linear models (GLM) will be used for unequal cells. Various regression models (e.g. linear, curvilinear etc.) will be tested to look for trends of changes in sap chemistry in predictor variables i.e. sites and the healthy versus declining trees. Significant differences among chemicals or sites will be examined by Duncan's multiple range test. IBM 4381 Mainframe computer will be used for complex statistical procedures (for example GLM) using SAS under VM/CMS operating system. Basic and Fortran compilers will be used to further modify or reprogram the SAS procedures.

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